TRANSFORMATION PROTOCOL

MATERIAL

- SOC medium
- Distribution kit with the DNA in question
- 5X KCM buffer
- Competent cells
- Agar plates – plane and antibiotic of choice

PROCEDURE

1. Prepare following reagents and don't forget to prepare following devices:

2. Pre-heat rich SOC medium (with glucose) at 37°C under agitation,

3. Dissolve the DNA from the distribution Kit within the well in 10 µL of 5XKCM buffer by thoroughly pipetting up and down deep inside the well. Look to flush out each corner of the well and make clear that you have well re-suspended the DNA by a strong red colour.
Note: Use the RFP construct from the efficiency kit as a control.

4. Take out 1-2 µL of DNA solution and add it to 50µL of pre-cooled chemo-competent cells.

5. Add 10 µL of 5X KCM buffer to the competent cells in order to increase transformation efficiency.

6. Mix the solution well by pipetting up and down and very quick vortexing and spin down at the table centrifuge. The mixture should have a homogenous orange colour.

7. Incubate the solution on ice for 30 min. (Preheat meanwhile the heat block to 42°C)

8. Heat shock your solution for 1 min at 42°C.

9. Put the cells back on ice for 5 min to let them recover.

10. Add 200µL of pre-heated SOC medium to the cells and mix it carefully by pipetting up and down.

11. Incubate the cells for 2 hours at 37°C under permanent agitation.

12. Pre-heat agar plates with the corresponding antibiotic at 37°C in the incubator.

13. Add 200µL of your cell solution to the antibiotic plate and streak them out.

14. Add 20µL of your cell solution to a plane plate and streak them out.

15. Let the colonies grow overnight and pick them at the next day.